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Exploring Conformational Space and Dynamics of RNA Hairpins by MD Simulations: Structure-Function Correlation of HIV-1 Genome Regulatory Elements

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To assess the reliability of MD derived structural models of the dynamic multifunctional RNA hairpin, a comparison between back calculated from MD trajectories and experimentally observed proton chemical shifts has been carried out. Our results confirmed coexistence of alternative conformations of the apical loop of HIV-1 TAR RNA hairpin in a solution. The comparison between the calculated and observed proton chemical shifts proved to be a good tool for validation and refinement of MD derived structures of dynamically inter-converting RNA conformational sub states.

1 Introduction

Insight into dynamical behavior of RNA molecules by experimental techniques is limited. Elucidating high-resolution RNA structure of dynamically inter-converting conformational sub states poses significant challenges to NMR spectroscopy and X-ray crystallography, which remain limited in applicability to static average structure determination of well folded conformations. Functionally active RNA conformations may not always be most populated in a solution. Transiently populated conformational sub states may be captured during protein recognition and stabilized by binding divalent ions or other biomolecular complex components. To elucidate structure-function relationships in RNA, it is necessary to go beyond the static structure and understand the dynamics of RNA in a solution. Molecular modeling and molecular dynamics simulation (MD) methods complement limitations of experimental techniques. However, the determination of RNA conformational space by simulations is related to limited time and conformation range, which may be probed by MD in comparison to real RNA dynamics. Roughness of the folding energy surface, kinetic traps and stable misfolds are the factors which require attention not only during in vitro RNA folding procedures, but also during computer simulations. Adequately, special consideration of infrequent events in structural transitions is needed for MD experiments due to relatively low statistics reachable in current status of software and hardware available for these calculations. In our exploring of the conformational space accessible to the dynamic RNA hairpin by molecular modeling and extensive MD simulations, we adopted several alternative approaches, which revealed the coexistence of two stable conformers. Then we investigated whether our MD derived structural models agree with the experimental NMR parameters.

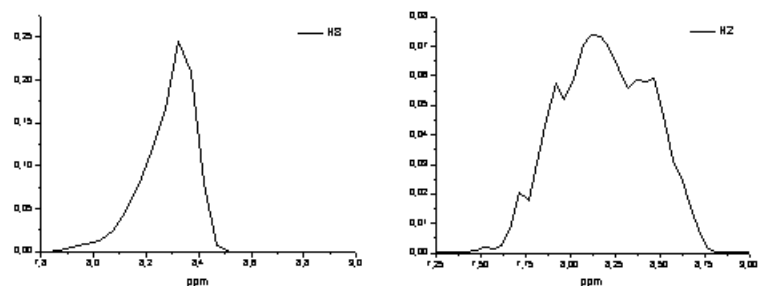


Figure 1. The distribution of CCS back-calculated from MD trajectories for H8 and H2 protons of adenosine A35. Experimentally measured values are 8.33 and 8.16, respectively.

2 Alternative Conformations of the Apical Loop of HIV-1 TAR RNA Hairpin

TAR RNA hairpin located in the extremely conserved domain of the 5' untranslated leader region (5' UTR) of the HIV-1 genome is involved in many steps of the virus replication cycle. Its functional diversity as viral regulatory element is determined by structural versatility. The structure of the TAR apical loop has been addressed by several experimental and computational methods. NMR studies revealed a compact loop structure with considerable conformational flexibility resulting in ensemble of NMR structural models¹. Structural studies by biochemical methods (UV melting and thermodynamics, chemical structure probing, lead induced cleavage) of TAR RNA hairpins with a selectively mutated or 2-aminopurine substituted apical loops demonstrated that the apical loop of TAR is structured and we postulated that it is stabilized by the C30 - G34 cross-loop base pair². Our recent molecular modeling and MD simulation studies revealed the coexistence of alternative conformations of the TAR apical loop: one stabilized by the C30 - G34 cross-loop base pair and the other stabilized by the interactions characteristic for U-turn motif³.

3 Validation of MD Derived Structural Models by Comparing the Calculated and Experimentally Observed NMR Data

Initial models built by fitting TAR sequence to selected RNA motifs were adjusted by imposing constrains for the specific intra-loop interactions during short MD runs. All constraints were then removed and several full MD trajectories were collected running simulations for up to 8 ns each, depending on the convergence of the simulated structures, at different temperatures: 300K, 320K, 340K and 350K. The cluster analysis of trajectories yielded a range of conformational motifs characterized by different internal interactions and exhibiting diverse stability and convergence within the MD trajectories in a total of over 100 ns of simulation time. The reliability of the structural ensembles obtained during extensive MD simulations was assessed by NMR chemical shifts (CCS) calculations. CCS were back-calculated from the structures with the program NUCHEMICS, considering ring-current effects, magnetic-anisotropy terms, and ignoring charge contribution⁴. Each proton shift, calculated for structures within MD trajectories, contributed to the ensemble

of all calculated structures and was subsequently analyzed in ORIGIN in terms of statistical distribution. The structural analysis and interpretation of the TAR RNA 1H chemical shifts were carried out by comparing the calculated to experimental values⁵. Fig. 1 gives an example of the distribution of CCS back-calculated from MD trajectories for H8 and H2 protons of adenosine A35 base. Experimentally measured values are 8.33 and 8.16, respectively.

4 Conclusions

RNA hairpins exhibit remarkable versatility with regard to how conformational changes are utilized to modulate diverse cellular processes. RNA conformational switching is usually induced by cofactors such as proteins, small molecules, divalent ions or other RNAs. The study of the range of conformations accessible to RNA structural motifs is important for understanding the mechanisms of recognition processes and regulation by RNA. We observed good agreement between the back-calculated and experimental CCS for both MD derived structural models of the apical loop of TAR. Our results validate coexistence of both conformers of TAR RNA hairpin in a solution. Alternative structures are consistent with the range of previously obtained diverse experimental results. Both conformers can be functionally important: the conformer stabilized by cross-loop base pair can be responsible for TAR-proteins recognition, whereas the conformer stabilized by the interactions characteristic for U-turn motif can mediate RNA-RNA and RNA-DNA loop-loop interactions. The comparison between the calculated and observed CCS proved to be a good tool for validation and refinement of MD derived structures of dynamically inter-converting RNA conformational sub states.

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